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Interaction between an Ammonium-Oxidizer, 'Nitrosomonas' sp., and Two Heterotrophic Bacteria, 'Nocardia atlantica' and 'Pseudomonas' sp.: A Note

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16. ABSTRACT

Closed culture experiments showed that the ability of an estuarine chemolithotrophic ammonium-oxidizer, Nitrosomonas sp., to convert ammonium to nitrite was substantially increased by as much as 150% and 50% when grown in the presence of two heterotrophs isolated from the same environment. The heterotrophs were identified as Nocardia atlantica and Pseudomonas sp. The growth of the heterotrophs was stimulated by as much as one order of magnitude in the presence of the ammonium-oxidizer.

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Interaction Between an Ammonium-Oxidizer, *Nitrosomonas* sp., and Two Heterotrophic Bacteria, *Nocardia atlantica* and *Pseudomonas* sp.: A Note

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Abstract. Closed culture experiments showed that the ability of an estuarine chemolithotrophic ammonium-oxidizer, *Nitrosomonas* sp., to convert ammonium to nitrite was substantially increased by as much as 150% and 50% when grown in the presence of two heterotrophs isolated from the same environment. The heterotrophs were identified as *Nocardia atlantica* and *Pseudomonas* sp. The growth of the heterotrophs was stimulated by as much as one order of magnitude in the presence of the ammonium-oxidizer.

Note

It is well recognized but not especially well documented that nitrifying bacteria (ammonium-oxidizers and nitrite oxidizers) exhibit better growth and increased nitrification when heterotrophs are present in the cultures (3, 6, 11). However, there have been few attempts to measure the interactions between ammonium-oxidizers and heterotrophs from environmental sources or to identify the organisms involved. Furthermore, there is little information concerning the ecology of estuarine ammonium-oxidizers. The purpose of this paper is to describe the interactions between an ammonium-oxidizer and two heterotrophic bacteria which were isolated from an estuarine bay.

During every attempt to isolate the predominant ammonium-oxidizers from estuarine water and sediment samples, two heterotrophic bacteria were constantly present in the enrichment culture of the nitrifiers. One was a gram-positive pleomorphic rod which produced an orange pigment when grown on Eugonagar (BBL) and was identified as *Nocardia atlantica* (2, 7, 10). The other was a gram-negative rod which was identified as a species of *Pseudomonas* (10). The ammonium-oxidizer was isolated using ammonium-oxidizer medium (9) in continuous flow culture followed by serial dilutions and was identified by morphological characteristics and cytomembrane structure (12) as *Nitrosomonas* sp. A more complete description of the organism and the isolation technique has been described by the authors (manuscript submitted).

Mixed cultures of the organisms were prepared. The heterotrophic isolates were

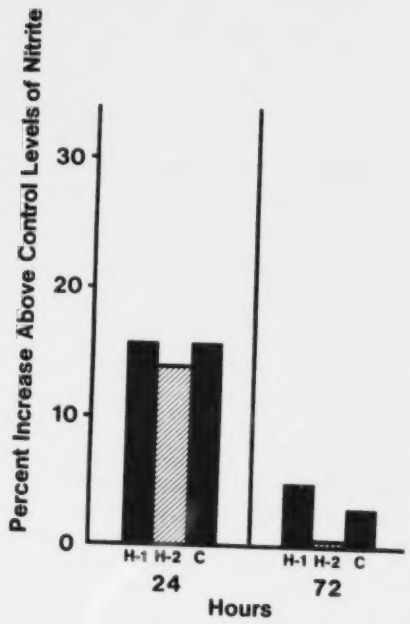

Fig. 1. Nitrite production by *Nitrosomonas* sp. in the presence of *Nocardia atlantica* (H-1), *Pseudomonas* sp. (H-2), and a combination of both (C). The inocula were 10^7 cells/ml. Control levels of nitrite were 12.0 mg/l at 24 h and 131.2 mg/l at 72 h.

grown in Eugonbroth (BBL), harvested by centrifugation at 2000 rpm, washed, and suspended in ammonium-oxidizer medium at a density of 10^7 cells/ml. The ammonium-oxidizer was grown in carbonate-free ammonium-oxidizer medium, harvested, and resuspended in fresh medium at a density of 10^7 cells/ml. These suspensions were inoculated into culture tubes containing 10 ml of ammonium-oxidizer medium with 5 g/l $CaCO_3$, pH 8.2, to give the following combinations: 1 ml *Nitrosomonas* sp. (N) and 1 ml sterile medium; 1 ml *Nocardia atlantica* (H-1) and 1 ml medium; 1 ml *Pseudomonas* sp. (H-2) and 1 ml medium; 0.5 ml H-1, 0.5 ml H-2, and 1 ml medium; 1 ml N and 1 ml H-1; 1 ml N and 1 ml H-2; 1 ml N, 0.5 ml H-1, and 0.5 ml H-2. The cultures were shaken in a 30°C incubator. The pH was checked at 24-hour intervals. Nitrite was assayed using the method described by Bendschneider and Robinson (1). Three replicate tubes were prepared, and the experiment was performed twice.

The effects of the heterotrophs on ammonium oxidation, shown in Fig. 1, indicate that their presence stimulated ammonium-oxidation by 15% and 12% at 24 hours. *Nocardia atlantica* appeared to stimulate ammonium oxidation at a greater rate than the *Pseudomonas* sp., while the combination of the two isolates resulted in an increase in ammonium oxidation such that a linear relationship between activity and the initial cell concentration was indicated. In the combined cultures, activity was one-half the activity of H-1 plus one-half the activity of H-2, the same relationship of the initial inocula.

To ensure that heterotrophic nitrification was not occurring, nitrite was assayed in cultures containing only the heterotrophs and in cultures containing the heterotrophs plus added nutrients (0.1% peptone, yeast extract). Since no nitrite was detected, it can be assumed that nitrification was carried out only by the chemolithotrophic bacteria.

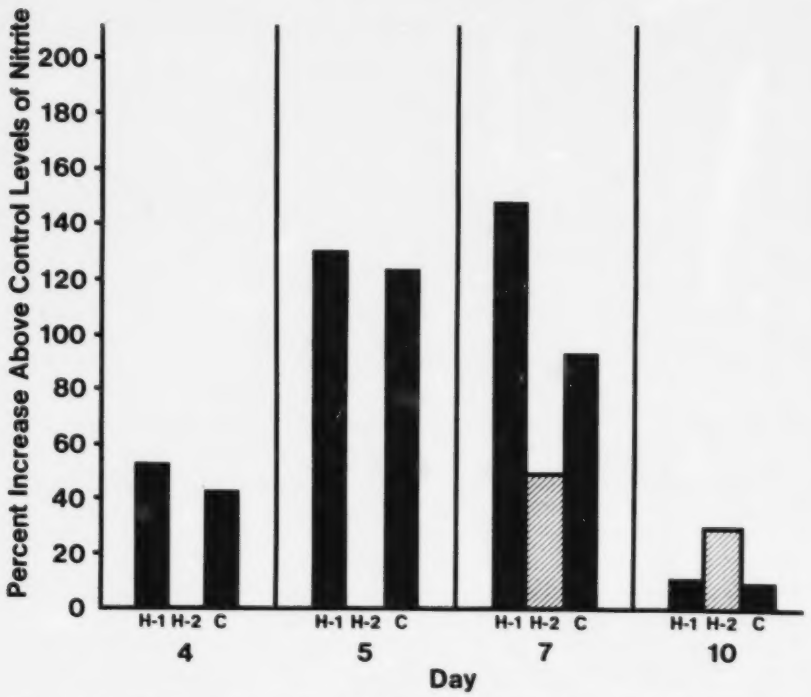

Fig. 2. Nitrite production by *Nitrosomonas* sp. in the presence of *Nocardia atlantica* (H-1), *Pseudomonas* sp. (H-2), and a combination of both (C). The inocula were 10^2 cells/ml. Control levels of nitrite were 0.33 mg/l at 4 days, 0.91 mg/l at 5 days, 36.1 mg/l at 7 days, and 290.0 mg/l at 10 days.

To examine the interactions of the ammonium-oxidizer and the heterotrophs using cell populations more similar to those found in the environment, the inocula were diluted with sterile medium to give 10^2 cells/ml. The same procedure was followed as previously described with similar replications. Standard plate counts using Eugonagar were performed on days 0, 1, 3, 4, 5, 7, and 10. After 48 hours of incubation at 30°C all colonies were counted. Standard plate counts were replicated using three dilutions.

The results are shown in Figs. 2 and 3. Nitrite was first detected on day 4. A substantial increase in ammonium oxidation was observed in the *Nitrosomonas* sp. cultures containing isolate H-1, especially at day 7 when nitrite levels were 150% greater than concentrations in the controls. Ammonium oxidation was also stimulated at days 7 and 10 by isolate H-2. Ammonium oxidation in the cultures containing both heterotrophs appeared to reflect an activity similar to that of H-1.

The effect of the ammonium-oxidizers on the growth of the heterotrophs is presented in Fig. 3. Both isolates showed increased growth in the presence of the ammonium-oxidizers, especially isolate H-1. The heterotrophs seem to exhibit best growth after 7 days. It was interesting to note that in the cultures containing the ammonium-oxidizers

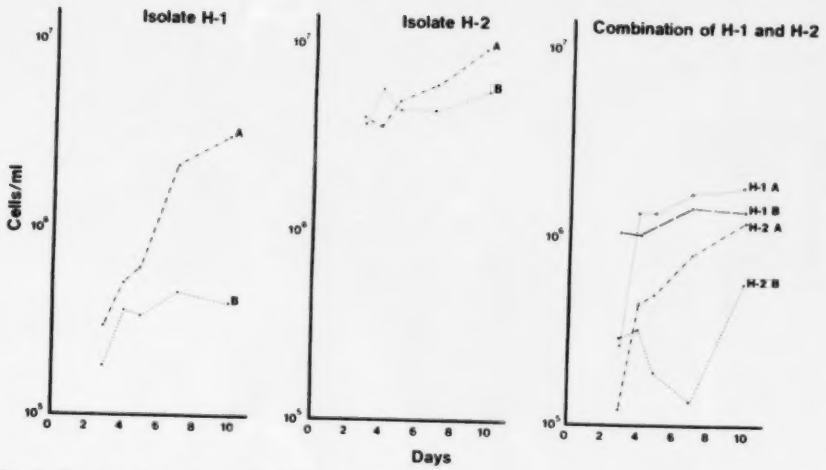

Fig. 3. Growth of *Nocardia atlantica* (H-1), *Pseudomonas* sp. (H-2), and a combination of both isolates in ammonium-oxidizer medium with (A) and without (B) *Nitrosomonas* sp.

and both heterotrophs, growth of each isolate was not stimulated as greatly as when each organism was grown separately with the ammonium-oxidizer.

The results indicate that there are significant interactions between environmental strains of the ammonium-oxidizer, *Nitrosomonas* sp., and the heterotrophs, *Nocardia atlantica* and *Pseudomonas* sp. in mixed culture. Previous work by Clark and Schmidt (3) has shown that stimulation of ammonium oxidation occurred when *Nitrosomonas europaea* was grown in the presence of a contaminating heterotrophic organism whose source was unknown. However, they found that the increase in nitrite production coincided with a decrease in the heterotrophic population. They attributed the increase in nitrite production to compounds which were released upon lysis of the heterotrophic cells. Such a relationship was not observed in the cultures examined here. Both the heterotrophs and ammonium-oxidizer appeared to benefit significantly in the mixed cultures over an extended time period, especially at cell concentrations similar to those in the environment (10^2 cells/ml). Since several vitamins, amino acids, and various other organics have been shown to stimulate ammonium oxidation (4, 5, 8), it is possible that the heterotrophs may be producing one or more products which stimulate the growth of the ammonium-oxidizers and thus increase ammonium oxidation. In turn, the ammonium-oxidizers which fix C may release organic carbon which supports heterotrophic growth. Several other factors, notably pH and CO_2, might be suspected of stimulating ammonium oxidation. However, the pH changes that occurred in the controls and in the mixed cultures were not significantly different to affect ammonium oxidation. Furthermore, the calcium carbonate buffer provides ample CO_2 for the ammonium oxidizers such that any CO_2 produced by the heterotrophs would not stimulate the chemolithotrophs. Further studies using continuous flow culture, however, are warranted to identify and better understand these factors. Whether the interactions described here actually occur between the organisms in the estuarine environment is

difficult to speculate, but since the organisms occur in the same environment and seem to be found in close proximity, they may exhibit such a mutualistic relationship in situ.

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